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A COMPARATIVE STUDY REGARDING THE ASSOCATION OF ALPHA-2U GLOBULIN WITH THE NEPHROTOXIC MECHANISM OF CERTAIN PETROLEUM-BASED AIR FORCE FUELS

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Fischer 344 male rats have a dose and time-dependent renal proximal tubular degeneration induced by certain hydrocarbon compounds. This degeneration may be associated with a low molecular weight urinary protein called alpha-2U globulin. We are using rat strain variation of the alpha-2U globulin molecule and metabolic alteration of the urinary pH as methods to investigate the hydrocarbon-induced nephrotoxic response. Two significant advances have been made in the first year of this project: (1) We have developed a histochemical procedure to specifically evaluate changes in the renal epithelial lysosome, and (2) we have detected a rat strain difference in susceptibility to hydrocarbon-induced nephrotoxicity.

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AFOSR 88-0033

Annual Technical Report (12/1/87-11/30/88)

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December, 1988

Summary

Fischer 344 male rats have a dose and time-dependent renal proximal tubular degeneration induced by certain hydrocarbon compounds. This degeneration may be associated with a low molecular weight urinary protein called alpha-2U globulin. We are using rat strain variation of the alpha-2U globulin molecule and metabolic alteration of the urinary pH as methods to investigate the hydrocarbon-induced nephrotoxic response. Two significant advances have been made in the first year of this project: (1) We have developed a histochemical procedure to specifically evaluate changes in the renal epithelial lysosome, and (2) we have detected a rat strain difference in susceptibility to hydrocarbon-induced nephrotoxicity.

Introduction

The exposure of male rats to certain hydrocarbon compounds (e.g. decalin) results in a nephrotoxic response. The human renal response to hydrocarbon exposure is less clearly defined. Most studies have focused on leaded gasoline and have failed to demonstrate any consistent association between long term, low level agent exposure and renal disease. However, the potential human health hazard of environmental or occupational hydrocarbon exposure has warranted continued epidemiological and mechanistic studies. Although the primary animal model for human risk assessment of hydrocarbon compounds is the rat, there is considerable controversy regarding the validity of this model. The basis for the controversy is centered on a urinary protein called alpha-2U globulin (A2U), which appears to be unique to the rat.

The principal investigator, in collaboration with toxicologists at AAMRL/THT, Wright-Patterson AFB, is studying the association of A2U with the hydrocarbon-induced nephrotoxic process. The principal objectives of this study are:

(I) To evaluate the relative sensitivities of albino and pigmented rat strains to hydrocarbon-induced nephrotoxicity.

(II) To evaluate hydrocarbon-induced nephrotoxicity after genetic modification of A2U by interstrain breeding.

(III) To compare metabolic alteration of urinary pH with the occurrence of nephrotoxicity.

The projected time course to complete the above objectives is from December 1, 1987 through November 30, 1989. This progress report represents our efforts from December 1, 1987 through December 31, 1988.

Status of the Research

Objective (I)

Experiment #1: Strain susceptibility to decalin exposure-Trial #1
Fischer 344 albino male rats: 4 control and 8 experimental animals.
Long-Evans pigmented male rats: 4 control and 8 experimental animals.
Fawn-Hooded pigmented male rats: 4 control and 8 experimental animals.

Urinary A2U

We have confirmed our previous findings (Eurell et al., 1988) regarding the molecular heterogeneity of urinary A2U from different rat strains. We noted an increased concentration of urinary A2U in all experimental animals (relative to control animals). Electrophoretic analysis of the A2U molecule revealed minor differences in the A2U isoelectric variants between different strains of experimental animals following decalin exposure. We are continuing our analysis of the A2U molecule.

Histopathology (H&E stain)

Standard Hematoxylin and Eosin staining techniques revealed a marginal difference in the lysosomal accumulation of renal proximal epithelial cells between the albino and pigmented rat strains (Tables I-III). The Fawn/Hooded rat appeared to be less susceptible to the nephrotoxicity than either the Fischer 344 or the Long/Evans strain.

Histopathology (Naphthol AS-TR phosphate-Hexazonium stain)

We believe that the Hematoxylin and Eosin technique may not be sufficiently sensitive to detect subtle strain differences in the nephrotoxic response. In order to increase the sensitivity of our histopathologic analysis of lysosomal changes in renal cells we have developed a Naphthol AS-TR phosphate-Hexazonium salt-based stain for acid phosphatase (a lysosomal marker). This procedure reveals striking differences between the lysosomes of control and experimental animals (Figures 1 and 2). Quantitative histochemical techniques are currently being developed to verify the morphologic data.

Table 1. Pathology summary sheet following decalin exposure.

Fischer 344 Male Rat

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
88-1	+/-	+/-	-	-	-
88-2	+/-	+/-	-	-	-
88-3	+/-	+/-	1	-	-
88-4	+/-	+/-	-	-	-
DECALIN (1.0 ML/KG)					
88-5	2	1	1	OCC. (H)	#2
88-6	2	1	+/-	-	-
88-7	2	+/-	1	-	#1
88-8	2	+/-	1	-	#1
DECALIN (2.0 ML/KG)					
88-9	2-3	1	1-2	1 (H/C)	#1
88-10	2	1	1-2	1 (H/C)	#1
88-11	3	+/-	2	1 (H/C)	#1,#3
88-12	2	+/-	1	-	#1

NOTE:OCC.=OCCASIONAL;(H)=HYALINE;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI.REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES; #3=CORTICAL TUBULAR NEPHROLITHS (BASOPHILIC OVOID BODIES)

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

Table 2. Pathology summary sheet following decalin exposure.

Long/Evans Male Rat

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
88-13	+/-	+/-	-	-	-
88-14	+/-	-	-	-	-
88-15	+/-	+/-	-	-	-
88-16	+/-	-	-	-	-
DECALIN (1.0 ML/KG)					
88-17	2	+/-	1	-	#1
88-18	2-3	+/-	+/-	OCC. (H)	#1
88-19	2	1	1	-	#1
88-20	2	+/-	1	OCC. (H/C)	#1,#2
DECALIN (2.0 ML/KG)					
88-21	2-3	2	1-2	2 (H/C)	#1,#2
88-22	2	1	1-2	1 (H/C)	#1
88-23	2	1	2	1 (H/C)	#1
88-24	2-3	1	2	2 (H)	#1

NOTE:OCC.=OCCASIONAL;(H)=HYALINE;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI.REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES.

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

Table 3. Pathology summary sheet following decalin exposure.

Fawn/Hooded Male Rat

TREATMENT	HYALINE DROPLETS IN PROX. EPI.	CORTICAL TUBULAR DILATION	PROXIMAL TUBULAR EPI. NECROSIS	CASTS	OTHER
H2O (2.0 ML/KG)					
88-25	+/-	-	-	-	-
88-26	1	-	-	-	-
88-27	+/-	+/-	-	-	-
88-28	+/-	+/-	-	-	-
DECALIN (1.0 ML/KG)					
88-29	1	+/-	+/-	-	#1
88-30	1-2	-	+/-	-	#1
88-31	2	1	1	OCC. (H/C)	#1,#2
88-32	1-2	+/-	1	-	#1
DECALIN (2.0 ML/KG)					
88-33	1-2	1	1-2	-	#1,#2
88-34	2	1	1	OCC. (H/C)	#1
88-35	2	1	2	-	#1
88-36	2	+/-	2	-	#1,#2

NOTE:OCC.=OCCASIONAL;(H)=HYALINE;(H/C)=HYALINE/CELLULAR; #1=PROXIMAL TUBULAR EPI.REGENERATION; #2=FOCAL PERIVASCULAR LYMPHOID AGGREGATES.

SCORING- (+/-)=MINIMAL; (1)=MILD; (2)=MODERATE; (3)=SEVERE

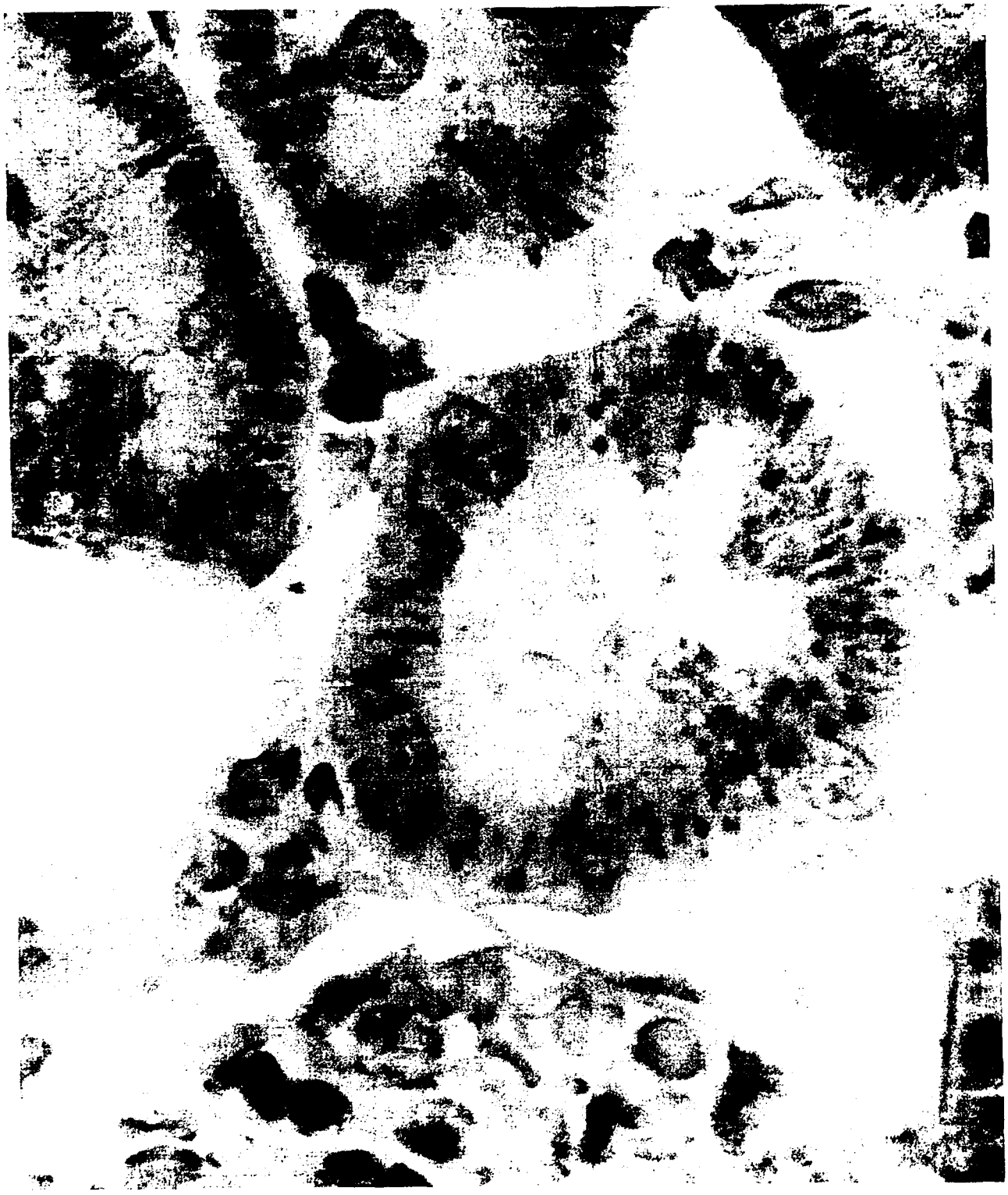

Figure I. Naphthol AS-TR phosphate-Hexazonium salt-based stain for lysosomal acid phosphatase. Control Fischer 344 male rat (2ml/kg H20). Note small, dense, reddish-staining lysosomes in renal tubular epithelial cells. Methyl green=nuclear counterstain.

Figure II. Naphthol AS-TR phosphate-Hexazonium salt-based stain for lysosomal acid phosphatase. Experimental Fischer 344 male rat (2ml/kg Decalin). Note large, swollen, pale orange lysosomes in renal tubular epithelial cells. Methyl green=nuclear counterstain.

Approximately 50% of the Objective (I)-Experiment #1 samples have been analyzed using the Naphthol AS-TR phosphate-Hexazonium salt-based staining method. Preliminary results indicate that the Fawn\Hooded strain is less sensitive to the nephrotoxic effect than the Fischer 344 strain. The Long/Evans strain appears to be equally sensitive to the nephrotoxicity as the Fischer 344 strain. The Naphthol AS-TR phosphate-Hexazonium salt-based staining method has two advantages over the standard H&E procedure: (1) a clear difference in the target organelle of experimental and control animals, and (2) the ability to apply quantitative histochemical techniques to kidney samples, thereby allowing a more objective evaluation of the nephrotoxic response.

Experiment #2: Susceptibility of Fischer 344 male rats to different hydrocarbon agents-Trial#1

Decalin: 1 control + 3 experimental
Trimethylpentane: 1 control + 3 experimental
Dimethylmethylphosphonate: 1 control + 3 experimental
JP-10 fuel: 1 control + 3 experimental

This preliminary experiment was run to support both the objectives of this research project and an ongoing project at AAMRL/THT, Wright-Patterson AFB (Dr. D. Mattie, P.I.). The histochemical technique to evaluate lysosomal morphology recently developed in Dr. Eurell's laboratory has provided a new tool to compare the hydrocarbon-induced nephrotoxic response using both light and electron microscopic techniques. At the time of this report approximately 20% of the samples have been processed.

Objective (II)

Interstrain breeding effect on nephrotoxicity-Trial #1
F344 x L-E male rats: 3 control and 9 experimental animals
F344 x F-H male rats: 3 control and 9 experimental animals

Samples recovered from interstrain animals analyzed by the standard H&E technique appeared to show the same degree of nephrotoxic change (see criteria, Table #1) as the F344 strain. We plan to begin processing the interstrain kidney samples with the Naphthol AS-TR phosphate-Hexazonium salt-based stain in mid-January 1989. We believe this will provide the best chance of detecting a subtle difference which might exist.

Objective (III)

Metabolic alteration of urinary pH-Trial #1
Fischer 344 male rats
3 control animals and 6 experimental animals

Metabolic alteration of urinary pH-Trial #2
Fischer 344 male rats
4 control animals and 20 experimental animals

Preliminary studies using methods referenced in the literature failed to significantly alter the urinary pH of experimental animals.

Future Plans

Our analysis to date, suggests that the strain differences seen in the urinary A2U molecule are reflected in the renal cell lysosomal changes following decalin exposure. We will test this finding using quantitative histochemical techniques.

We are currently attempting to refine our method for the isolation of alpha-2U globulin isotypes using a combination of preparative isoelectric focusing and chromatofocusing techniques.

We have conferred with a colleague in renal physiology and will begin a new series of metabolic alteration studies in February-March, 1989.

Publications

Eurell, T.E., Parnell, M.J., and Henningsen, G.M. Comparison of A2U globulin isolated from the urine of albino and non-albino male rats. The Toxicologist, (8:1):#536, February, 1988.

Eurell, T.E., Parker, R.D., and Alden, C.L. Lysosomal changes in renal tubular epithelial cells of male Sprague-Dawley rats following decalin exposure. Accepted for Poster/Discussion 28th Annual Meeting of the Society of Toxicology, Atlanta, Georgia, Feb. 27-Mar.3, 1989.

Eurell, T.E., Parnell, M.J., and Henningsen, G.H. Comparison of alpha-2U globulin isolated from the urine of albino and non-albino male rats. (in preparation).

Interactions (Coupling Activities)

Dr. Eurell conferred with Dr.David Mattie and Ms. Marylyn George at AAMRL/THT, Wright-Patterson AFB on November 2, 1988. During that visit Dr. Eurell presented an update on this project and planned the next set of experiments in collaboration with AAMRL/THT staff.